Open Access
Asian Australas. J. Anim. Sci.
Vol. 27, No. 5 : 700-705 May 2014
http://dx.doi.org/10.5713/ajas.2013.13616

www.ajas.info
pISSN 1011-2367 eISSN 1976-5517

Effect of β-Mannanase and α-Galactosidase Supplementation to Soybean Meal Based Diets on Growth, Feed Efficiency and Nutrient Digestibility of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum)

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ABSTRACT: A 12-week feeding trial was conducted with 87 g rainbow trout to evaluate the effects on growth performances, feed efficiency and nutrient digestibility of adding β-mannanase and α-galactosidase enzymes, solely or in combination. Seven diets were prepared by adding β-mannanase, α-galactosidase and mixed enzyme at two different levels (1 g/kg and 2 g/kg) to control diet (without enzyme) including soybean meal. Mixed enzymes (1 g/kg, 2 g/kg) were prepared by adding β-mannanase and α-galactosidase at the same doses (0.5+0.5 g/kg and 1+1 g/kg). At the end of the experiment, addition of β-mannanase, α-galactosidase and mixed enzyme to diet containing 44% soybean meal had no significant effects on growth performance and gain:feed ($p>0.05$). In addition, adding β-mannanase, α-galactosidase and mixed enzyme in different rations to trout diets had no affect on nutrient digestibility and body composition ($p>0.05$). (**Key Words:** Rainbow Trout, *Oncorhynchus mykiss*, Soybean Meal, Growth, β-Mannanase, α-Galactosidase, Enzyme)

INTRODUCTION

Soybean meal (SBM) is probably the most promising and most studied alternative protein source to fish meal. However, SBM is low in some amino acids and contains antinutritional factors (Tacon 1995; El-Sayed, 1999; Krogdahl et al., 2010; Hagely et al., 2013). The SBM contains α-galactosides that cannot be digested in the intestine of monogastrics including fish, due to the absence of α-galactosidase (Refstie et al., 1998; Waldroup et al., 2006; Zhang et al., 2010; Sørensen et al., 2011; Hagely et al., 2013). In addition, SBM contains approximately 13 to 20 g/kg β-mannans (Dierick 1989; Mehri et al., 2010; Mok et al., 2013). β-Mannans, mainly exist in fiber fractions of soybean meal, and are highly viscous and intensely anti-nutritional (Kong et al., 2011). Previous studies reported that β-mannans in feed reduced nutrient digestibility in monogastric animals (poultry and swine) (Ritz et al., 1995; Dusel et al., 1997; Bedford and Schulze, 1998; Yamka et al., 2005). The use of exogenous enzymes to increase the digestibility of oligosaccharides has shown positive results in some terrestrial species (Ghazi et al., 2003; Wan et al., 2005; Waldroup et al., 2006; Zang et al., 2010).

Generally, while carbohydrase enzymes supplementation is common as commercial practice in swine and poultry industries, the use of these enzymes has not received much attention in aquaculture. To date, a few studies have been conducted on the addition of carbohydrate degrading enzymes to fish diets (Ng and Chong, 2002; Zamini et al., 2012).

Therefore, this study was conducted to examine the effects of adding β-mannanase, α-galactosidase and mixed enzyme at two different levels to soybean meal on growth performances, gain:feed, nutrient digestibility and body composition in rainbow trout.

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Submitted Sept. 30, 2013; Revised Dec. 2, 2013; Accepted Feb. 18, 2014

MATERIALS AND METHODS

Diet preparation and analysis

Experimental diets were isonitrogenous (40% crude protein [CP]) and isoenergetic (17.35 MJ/kg) (Table 1). Experimental diets were formulated to fulfill the nutritional requirements of rainbow trout (NRC, 2011). Seven diets were prepared by adding β-mannanase, α-galactosidase and mixed enzyme at two different levels (1 g/kg and 2 g/kg) to control diet (without enzyme) which included soybean meal. Mixed enzymes (1 g/kg, 2 g/kg) were prepared by adding β-mannanase and α-galactosidase at the same ratio (0.5+0.5 g/kg and 1+1 g/kg).

These inclusion levels of enzymes were recommended by feed companies. β-Mannanase (Hemicell-L, 720,000 units/mL) and α-galactosidase (15, 000 unit/g) was supplied by Chemgen Corporation (Gaithersburg, MD, USA) and Ultra Bio-Logics Inc. (Châteauguay, QC, Canada), respectively. In the experiment, dehulled, toasted and solvent extracted SBM was used. The feed ingredients were supplied from a local fish feed manufacturer. All ingredients were ground to small particle size (0.5 mm) in a mill. Enzymes and micro ingredients were first mixed and then slowly added to the macro ingredients to ensure a homogenous mixture. Water was added to obtain 25% moisture level. Diets were passed through a mincer with a 1 cm sieve. The spaghetti-like strands were dried at 20°C for 16 hours in a convection oven. After drying, the diets were broken into 1 cm pellets. All pelleted diets were stored at 4°C until used. Ingredient composition and proximate analysis of the diets are given in Table 1. The moisture, CP, crude fiber and ash contents of the experimental diets, feces samples and body composition were determined according to standard AOAC methods (AOAC, 2002). The total lipids of all samples were determined by the chloroform-methanol extraction method (Bligh and Dyer, 1959).

Experimental animal and culture conditions

Feeding trial was conducted in Egirdir Fisheries Faculty of the Suleyman Demirel University (Turkey). Rainbow trout (*Oncorhynchus mykiss*) were obtained from a commercial fish farm situated in Turkey. Forty five rainbow trout (initial mean body weight 87.31±0.47 g) were stocked into each of 21 fibre tanks. The experiment was arranged as a completely randomised design of 7 treatments×3 replicates, each with 45 trout. Each tank was supplied continuously with spring water. The rainbow trout were fed by hand twice (09:00 and 16:00 hours) per day. Fish were fed according to 2.5% of body weight (BW) and water temperature. Each treatment was divided in to triplicate groups. The feeding trial was conducted for 12 wk. At the end of the experiment, five fish from each tank were killed and frozen at −20°C for body composition analysis. Water quality parameters such as temperature, dissolved oxygen level and pH were determined as 11±0.5°C, 7.27±0.8 mg/L, 7.12±0.2 throughout the experiment.

Growth performance analysis

The growth parameters were calculated using the following formulas. Mean BW was used to calculate growth

Table 1. Formulation of the experimental diets and proximate analysis

Ingredients (g/kg)	Trial groups [1]						
	Cont.	Man. (1 g/kg)	Man. (2 g/kg)	Gal. (1 g/kg)	Gal. (2 g/kg)	Mix (1 g/kg)	Mix (2 g/kg)
Fish meal (65%)	31.00	31.00	31.00	31.00	31.00	31.00	31.00
Soybean meal (44%)	44.00	44.00	44.00	44.00	44.00	44.00	44.00
Wheat flour	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Corn flour	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Soybean oil	11.70	11.70	11.70	11.70	11.70	11.70	11.70
Vit-Min[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Pellet binder	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Enzyme	0.0	0.1	0.2	0.1	0.2	0.1	0.2
Chemical composition (DM)							
DM (%)	92.75	93.62	93.23	92.94	92.39	93.48	92.47
CP (%)	41.17	40.39	40.94	40.72	40.88	41.74	40.02
Total lipid (%)	18.66	19.39	19.53	19.84	19.29	18.39	19.10
Crude fiber (%)	2.39	2.68	2.92	2.57	2.76	2.93	2.56
Crude ash (%)	11.44	10.52	10.72	10.88	10.64	11.28	10.48
DE (MJ)	17.35	17.35	17.35	17.35	17.35	17.35	17.35

DM, dry matter; CP, crude protein; DE, digestible energy.

[1] Cont., control, Man., β-mannanase, Gal., galactosidase, Mix, β-mannanase+galactosidase.

[2] Provided the following per kg diet: 20,000 IU vitamin A; 2,400 IU vitamin D_3; 12 mg vitamin E; 12 mg vitamin K_3; 20 mg vitamin B_1; 30 mg vitamin B_2; 2 mg Niacin; 50 mg Cal.D. Pantothenate; 20 vitamin B_6; 0.05 mg vitamin B_{12}; 0.5 mg D-Biotin; 6 mg folic acid; 200 mg vitamin C; 300 mg inositol, Mn; 118 mg as manganese sulfate; Zn, 375 mg as zinc oxide; Cu, 15 mg copper as copper sulfate; Co, 10 mg as cobaltous carbonate; I, 13.75 mg as potassium iodate; Se, 0.50 mg as sodium selenite; Mg, 10 mg as magnesium oxide.

parameters.

Weight gain (WG) (g) = (final BW, g)−(initial BW, g)

Specific growth rate (SGR) (%/d)
= [(ln final BW)−(ln initial BW)/days]×100

Gain:feed
= [(final BW, g)−(initial BW, g)]
/total feed intake [dry matter (DM), g]

Protein efficiency ratio (PER)
= (weight gain, g)/protein intake (g)

Survival rate (%)
= (Final fish number/initial fish number)×100

Digestibility trial

Apparent digestibility coefficients were measured by the indicator method using 0.5% chromic oxide as a marker (Hardy and Barrows, 2002). The collection of fish feces was carried out during the feeding trial. Fecal material collection was carried out manually by siphoning feces and straining through a fine-mesh-size net. After the last feeding at 09:00, the floor of tanks was siphoned to clean them. Feces were collected by siphon at 15:00 before the last feeding. Feces of each treatment were kept at −20°C and then oven dried at 50°C for 48 h and used in the analysis of chromic oxide and nutrients (Lim et al., 2001). Apparent digestibility coefficients (ADC) were calculated using the following equation;

ADC
= 100−{100×[Cr_20_3 in diet (%)/Cr_20_3 in feces (%)]
×(nutrient in feces (%)/nutrient in diet (%)}

Statistical analyses

Experiment was arranged as completely randomised design of 7 treatments by 3 replicates. Each tank was stocked with 45 fish with an initial average weight of 87 g. One-way ANOVA was used to compare growth parameters, gain:feed, nutrient digestibility and body composition among the treatments. All of the data were analyzed using SPSS for Windows. Duncan's multiple range test was used to determine the mean differences among the treatments (p = 0.05).

RESULTS

Growth performance data of the treatments are given in Table 2. Adding β-mannanase, α-galactosidase and mixed enzyme at different levels to trout diets had no affect on growth parameters and gain:feed (p>0.05). Weight gain, SGR, feed conversion ratio, and PER values ranged from 114.15 to 125.49, from 0.88 to 0.91, from 0.56 to 0.67, from 1.67 to 1.88, respectively. In addition, nutrient digestibility and body composition was not affected by enzyme adding at different levels to trout diets (Tables 3 and 4). The apparent DM, CP, and lipid digestibility ranged from 66.33% to 68.67%, from 83.33% to 86.67% and from 80.00% to 89.00%, respectively. There is no mortality during the research periods in experimental groups.

DISCUSSION

Effect of enzyme treatments on growth performance and feed efficiency

In the current experiment, addition of α-galactosidase enzyme at two different levels to SBM based diets did not affect on the growth performance and gain:feed of rainbow trout. There are a few studies conducted on the addition of α-galactosidase in fish diet. Farhangi and Carter (2007) informed that α-galactosidase enzyme supplementation to 50% dehulled lupin-based diet did not improve the growth performance and feed efficiency indices of rainbow trout. In contrast, Deguara et al. (1999) reported that the addition of the two enzyme mixes (protease+α-galactosidase) to the

Table 2. Growth parameters of rainbow trout fed diets supplemented with α-galactosidase and β-mannanase (p>0.05)

Growth parameters	Initial weight (g)	Final weight (g)	Weight gain (g)	SGR (%/d)	Gain/feed	PER
Cont.	90.02	204.00	114.15	0.91	0.56	1.83
Man. (1 g/kg)	87.31	203.00	115.59	0.94	0.63	1.79
Man. (2 g/kg)	88.33	208.30	120.18	0.95	0.60	1.67
Gal. (1 g/kg)	89.91	205.00	115.26	0.92	0.61	1.81
Gal. (2 g/kg)	86.13	205.67	119.47	0.97	0.56	1.79
Man+Gal. (1 g/kg)	86.27	211.67	125.49	0.98	0.67	1.88
Man+Gal. (2 g/kg)	88.09	206.89	118.22	0.94	0.62	1.87
SEM	1.33	3.73	3.69	0.02	0.06	0.06
P	0.27	0.71	0.41	0.53	0.86	0.34

SGR, specific growth rate; PER, protein efficiency ratio; Cont., control, Man., β-mannanase, Gal., galactosidase; SEM, pooled standard error of means. Data are means of three replicate tanks, each replicate was obtained from 45 fish.

Table 3. Apparent DM, CP, and total lipid digestibility coefficients (%) of rainbow trout fed α-galactosidase and β-mannanase supplemented soybean meal based diets (%) (p>0.05)

Groups	Parameter		
	DM	CP	Total lipid
Control	66.33	83.33	82.33
Man. (1 g/kg)	66.67	83.67	81.00
Man. (2 g/kg)	67.67	84.67	81.33
Gal. (1 g/kg)	64.00	83.33	80.00
Gal. (2 g/kg)	68.33	83.67	81.33
Man+Gal. (1 g/kg)	68.67	86.67	89.00
Man+Gal. (2 g/kg)	65.57	84.33	83.14
SEM	1.41	2.27	3.49
p value	0.31	0.94	0.59

DM, dry matter; CP, crude protein; SEM, pooled standard error of means. Data are means of three replicate tanks, each replicate was obtained from 45 fish.

320 g/kg SBM diet gave a very good improvement in performance of the gilthead sea bream (*Sparus aurata*) compared to fish fed the unsupplemented diet.

There are also a few studies conducted on the addition of β-mannanase in fish diet. Use of β-mannanase enzyme at two different levels (1 g/kg and 2 g/kg) to control diet including soybean meal in the current experiment did not affect the growth parameters and feed efficiency of rainbow trout. In contrast, Zamini et al. (2012) reported that addition of 0.5 g/kg and 2.5 g/kg β-mannanase to trade trout feeds resulted in significant improvements in body weight gain and feed efficiency in *Salmo trutta caspius*. In addition, 2.5 g/kg β-mannanase produced significantly higher growth than 0.5 g/kg β-mannanase and control diet. Differences may be due to different fish species used in these studies. Ng and Chong (2002) reported that addition of mix enzyme consisting of Allzyme Vegpro (protease and cellulose), Ronozyme (glucanase and pectinase) and pure β-mannanase to the 40% palm kernel meal diet did not improve growth and feed utilization of tilapia.

Table 4. Body composition of rainbow trout fed α-galactosidase and β-mannanase supplemented soybean meal based diets (%) (p>0.05)

Groups	Parameters			
	DM	CP	Total lipid	Crude ash
Control	62.22	21.03	5.26	1.76
Man. (1 g/kg)	61.38	19.65	4.60	1.95
Man. (2 g/kg)	66.39	19.92	5.93	1.73
Gal. (1 g/kg)	60.23	20.25	4.80	1.97
Gal. (2 g/kg)	58.95	19.08	5.01	1.96
Man+Gal. (1 g/kg)	63.67	20.71	4.41	2.02
Man+Gal. (2 g/kg)	63.45	20.17	4.25	1.80
SEM	4.40	0.81	0.73	0.13
p value	0.92	0.98	0.72	0.62

DM, dry matter; CP, crude protein; SEM, pooled standard error of means. Data are means of three replicate tanks, each replicate was obtained from 5 fish.

In the present study, mixed enzyme did not show significant improvements on the growth performance and feed utilization. There is no study of the effects on growth parameters by adding the combination of α-galactosidase and β-mannanase enzymes to SBM based diet for rainbow trout.

Effect of enzyme treatments on nutrient digestibility

In the present study, the addition of α-galactosidase, β-mannanase and mixed enzyme to diet containing SBM did not improve digestibility of protein, lipid and DM of trout. In contrast, Glencross et al. (2003) reported an improvement in protein digestibility in rainbow trout when lupin was treated with α-galactosidase. Farhangi and Carter (2007) also showed that α-galactosidase supplementation of a lupin based diet significantly improved CP digestibility, crude lipid and DM in rainbow trout.

Effect of enzyme treatments on body composition

Enzyme supplementation to SBM based diets in the current experiment did not influence the body composition. Similarly, Farhangi and Carter (2007) informed that enzymes supplementation did not affect carcass composition in rainbow trout. Ng and Chong (2002) indicated that addition of enzymes combination Allzyme Vegpro (protease and cellulose), Ronozyme (glucanase and pectinase) and pure β-mannanase supplementation in the diets had no effect on the whole body composition of tilapia.

In the present study, water temperature (11°C) might be one of the reasons to explain why growth performance and digestibility were not affected by enzyme supplementations. Forster et al. (1999) reported that the efficiency of dietary enzyme may be enhanced at higher water temperatures rather than low water temperatures. Vandenberg et al. (2012) also showed that efficiency of dietary phytase increased significantly when water temperature increased from 10 to 15°C.

CONCLUSION

This study is the first looking at the effect of α-galactosidase, β-mannanase and mixed enzyme supplementations to rainbow trout diet on growth and the apparent digestibility of nutrients in SBM based diet. The results of this experiment showed that growth parameters, gain:feed and PER were not affected by α-galactosidase, β-mannanase and mixed enzyme supplementation to 44% soybean meal based diet at 11°C. In addition, digestibility of DM, CP and lipid in rainbow trout was not affected by the supplementation of α-galactosidase, β-mannanase and mixed enzyme to soybean meal based diet.

ACKNOWLEDGMENTS

This study was supported for number of project (110O414) grant by TUBITAK, Scientific Technical Research Council in Turkey.

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